

What is claimed is:

1. A method of identifying a plurality of etiologic agents of disease in an individual comprising the steps of:

amplifying at least one nucleic acid molecule obtained from a biological sample
5 from the individual with a plurality of intelligent primers to obtain a plurality of amplification products corresponding to the plurality of etiologic agents; and

determining the molecular masses of the plurality of amplification products, wherein the molecular masses identify the plurality of etiologic agents and wherein the intelligent primers are broad range survey primers, division-wide primers, drill-down primers, or any
10 combination thereof.

2. A method of claim 1 wherein identification of at least one of the plurality of etiologic agents is accomplished at the genus or species level, and the intelligent primers are broad range survey primers, division-wide primers, or any combination thereof.

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3. A method of claim 1 wherein a subspecies characteristic of at least one of the plurality of etiologic agents is obtained using drill-down primers.

4. A method of claim 3 wherein the subspecies characteristic is serotype, strain type, sub-strain type, sub-species type, emm-type, presence of a bioengineered gene, presence of a toxin gene, presence of an antibiotic resistance gene, presence of a pathogenicity island, or presence of a virulence factor.

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5. A method of claim 1 wherein the molecular mass is determined by mass spectrometry.

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6. A method of claim 5 wherein the mass spectrometry is Fourier transform ion cyclotron resonance mass spectrometry (FTICR- MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF, or triple quadrupole.

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7. A method of claim 1 wherein the molecular masses are used to determine the base compositions of the amplification products and wherein the base compositions identify the pathogen.

8. A method of *in silico* screening of intelligent primer sets for identification of a plurality of bioagents comprising the steps of:

preparing a base composition probability cloud plot from a plurality of base composition signatures of the plurality of bioagents generated *in silico*;

5 inspecting the base composition probability cloud plot for overlap of clouds from different bioagents; and

selecting primer sets based on minimal overlap of the clouds.

9. A method of performing epidemic surveillance comprising the steps of:

10 amplifying at least one nucleic acid molecule obtained from a plurality of biological samples obtained from a plurality of geographic locations with at least one pair of intelligent primers to obtain at least one amplification product; and

determining the molecular mass of the at least one amplification product, wherein the molecular mass identifies the pathogen in the biological sample, and wherein
15 identification of a pathogen in a sample from a particular geographic location indicates the spread of the pathogen to the particular geographic location.

10. A method of claim 9 wherein the pathogen is a bacterium, a virus, a protozoan, a parasite, a mold, or a fungus.

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11. A method of claim 9 wherein the biological sample is blood, mucus, hair, urine, breath, saliva, sputum, stool, nail, or tissue biopsy.

12. A method of claim 9 wherein the biological sample is obtained from an animal.

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13. A method of claim 12 wherein the animal is a human.

14. A method of claim 12 wherein the intelligent primers are broad range survey primers, division-wide primers, or drill-down primers.

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15. A method of claim 14 wherein identification of the pathogen is accomplished at the genus or species level, and wherein the intelligent primers broad range survey primers or division-wide primers.

16. A method of claim 14 wherein a subspecies characteristic about the pathogen is obtained using drill-down primers.

17. A method of claim 16 wherein the subspecies characteristic is serotype, strain type, sub-strain type, sub-species type, emm-type, presence of a bioengineered gene, presence of a toxin gene, presence of an antibiotic resistance gene, presence of a pathogenicity island, or presence of a virulence factor.

18. A method of claim 9 wherein the molecular mass is determined by mass spectrometry.

19. A method of claim 18 wherein the mass spectrometry is Fourier transform ion cyclotron resonance mass spectrometry (FTICR- MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF, or triple quadrupole.

20. A method of claim 9 wherein the intelligent primers are targeted to ribosomal RNA or housekeeping genes.

21. A method of claim 9 wherein the molecular mass is used to determine the base composition of the amplification products and wherein the base compositions identify the pathogen.

22. A method for determining a subspecies characteristic of a pathogen in a biological sample comprising the steps of:

identifying the pathogen in the biological sample using broad range survey primers or division-wide primers;

selecting at least one pair of drill-down primers to amplify at least one nucleic acid segment which provides a subspecies characteristic of the pathogen;

amplifying the at least one nucleic acid segment to produce at least one drill-down amplification product; and

determining the base composition signature of the drill-down amplification product, wherein the base composition signature of the drill-down amplification product provides a subspecies characteristic of the pathogen.

23. A method of claim 22 wherein identification of the pathogen in the biological sample using broad range survey primers or division-wide primers comprises the steps of:

amplifying at least one nucleic acid molecule obtained from a biological sample with at least one pair of intelligent primers to obtain at least one amplification product,

5 wherein the intelligent primers are broad range survey primers or division-wide primers;

determining the molecular mass of the at least one amplification product; and

determining the base composition signature of the at least one amplification product,

wherein the base composition signature identifies the pathogen in the biological sample.

10 24. A method of claim 22 wherein the subspecies characteristic is serotype, strain type, sub-strain type, sub-species type, emm-type, presence of a bioengineered gene, presence of toxin gene, presence of antibiotic resistance gene, presence of a pathogenicity island, or presence of a virulence factor.

15 25. A method of pharmacogenetic analysis comprising the steps of:

amplifying a segment of genomic DNA obtained from an individual with at least one pair of intelligent primers to produce an amplification product, wherein the segment of genomic DNA provides pharmacogenetic information; and

determining the base composition signature of the amplification product, wherein

20 the base composition signature provides pharmacogenetic information about the individual.

26. A method of claim 25 wherein the pharmacogenetic information is a genetic metabolic disorder, a genetic defect in a receptor gene, or a single nucleotide polymorphism.

25 27. A method of identifying a pathogen in a biological sample comprising the steps of:

selecting a bioagent identifying amplicon;

selecting a pair of intelligent primers to obtain an amplification product of the bioagent identifying amplicon; and

determining the molecular mass of the amplification product, wherein said

30 molecular mass identifies the pathogen in the biological sample.

28. A method of claim 27 wherein the pathogen is a bacterium, a virus, a protozoan, a parasite, a mold, or a fungus.

29. A method of claim 27 wherein the biological sample is blood, mucus, hair, urine, breath, sputum, saliva, stool, nail, or tissue biopsy.

30. A method of claim 27 wherein the biological sample is obtained from an animal.

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31. A method of claim 30 wherein the animal is a human.

32. A method of claim 27 wherein the intelligent primers are broad range survey primers, division-wide primers, or drill-down primers.

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33. A method of claim 32 wherein identification of the pathogen is accomplished at the genus or species level, and wherein the intelligent primers are broad range survey primers or division-wide primers.

15 34. A method of claim 32 wherein a subspecies characteristic of the pathogen is obtained using drill-down primers.

35. A method of claim 34 wherein the subspecies characteristic is serotype, strain type, sub-strain type, sub-species type, emm-type, presence of a bioengineered gene, presence of a toxin gene, presence of an antibiotic resistance gene, presence of a pathogenicity island, or presence of a virulence factor.

20 36. A method of claim 27 wherein the molecular mass is determined by mass spectrometry.

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37. A method of claim 36 wherein the mass spectrometry is Fourier transform ion cyclotron resonance mass spectrometry (FTICR- MS), ion trap, quadrupole, magnetic sector, time of flight (TOF), Q-TOF, or triple quadrupole.

30 38. A method of claim 27 wherein the intelligent primers are targeted to ribosomal RNA or housekeeping genes.

39. A method of claim 27 wherein the molecular mass is used to determine the base composition of said amplification product and wherein said base composition identifies said pathogen.

5 40. An intelligent primer pair wherein each member of the pair has at least 70% sequence identity with the sequence of the corresponding member of any one of the following intelligent primer pair sequences: SEQ ID NOs: 8:9, 10:11, 12:13, 14:15, 16:17, 18:19, 20:21, 22:23, 24:25, 26:27, 28:29, 30:31, 32:33, 34:35, 36:37, 38:39, 40:41, 42:43, 44:45, 46:47, 48:49, 50:51, 52:53, 54:55, 56:57, 58:59, 60:61, 62:63, 64:65, 66:67, 68:69,
10 70:71, 72:73, 74:75, 76:77, 78:79, 80:81, 82:83, 84:85, 86:87, 88:89, 90:91, 92:93, 94:95, 96:97, 98:99, 100:101, 102:103, 104:105, 106:107, 108:109, 110:111, 112:113, 114:115, 116:117, 118:119, 120:121, 122:123, 124:125, 126:127, 128:129, 130:131, 132:133, 134:135, 136:137, 138:139, 140:141, 142:143, 144:145, 146:147, 148:149, 150:151, 152:153, 154:155, 156:157, 158:159, 160:161, 162:163, 164:165, 166:167, 168:169, 170:171, 172:173, 174:175,
15 176:177, 178:179, 180:181, 182:183, 184:185, 186:187, 188:189, 190:191, 192:193, 194:195, 196:197, 198:199, 200:201, 202:203, 204:205, 206:207, 208:209, 210:211, 212:213, 214:215, 216:217, 218:219, 220:221, 222:223, 224:225, 226:227, 228:229, 230:231, 232:233, 234:235, 236:237, 238:239, 240:241, 242:243, 244:245, 246:247, 248:249, 250:251, 252:253, 254:255, 256:257, 258:259, 260:261, 262:263, 264:265, 266:267, 268:269, 270:271, 272:273, 274:275,
20 276:277, 278:279, 280:281, 282:283, 284:285, 286:287, 288:289, 290:291, 292:293, 294:295, 296:297, 298:299, 300:301, 302:303, 304:305, 306:307, 308:309, 310:311, 312:313, 314:315, 316:317, 318:319, 320:321, 322:323, 324:325, 326:327, 328:329, 330:331, 332:333, 334:335, 336:337, 338:339, 340:341, 342:343, 344:345, 346:347, 348:349, 350:351, 352:353, 354:355, 356:357, 358:359, 360:361, 362:363, 364:365, 366:367, 368:369, 370:371, 372:373, 374:375,
25 or 376:377.

41. The intelligent primer pair of claim 40 comprising at least one modified nucleobase.

42. The intelligent primer pair of claim 41 wherein the modified nucleobase is 5-
30 propynylcytidine or 5-propynyluridine.

43. A bioagent identifying amplicon comprising an isolated polynucleotide of about 45 to about 150 nucleobases in length produced by the process of amplification of nucleic acid

from a bioagent with a pair of intelligent primers wherein each intelligent primer is of a length of about 12 to about 35 nucleobases, wherein the bioagent identifying amplicon provides identifying information about the bioagent.

5 44. The bioagent identifying amplicon of claim 43 wherein each member of the pair has at least 70% sequence identity with the sequence of the corresponding member of any one of the following intelligent primer pair sequences: SEQ ID NOs: 8:9, 10:11, 12:13, 14:15, 16:17, 18:19, 20:21, 22:23, 24:25, 26:27, 28:29, 30:31, 32:33, 34:35, 36:37, 38:39, 40:41, 42:43, 44:45, 46:47, 48:49, 50:51, 52:53, 54:55, 56:57, 58:59, 60:61, 62:63, 64:65, 66:67, 10 68:69, 70:71, 72:73, 74:75, 76:77, 78:79, 80:81, 82:83, 84:85, 86:87, 88:89, 90:91, 92:93, 94:95, 96:97, 98:99, 100:101, 102:103, 104:105, 106:107, 108:109, 110:111, 112:113, 114:115, 116:117, 118:119, 120:121, 122:123, 124:125, 126:127, 128:129, 130:131, 132:133, 134:135, 136:137, 138:139, 140:141, 142:143, 144:145, 146:147, 148:149, 150:151, 152:153, 154:155, 156:157, 158:159, 160:161, 162:163, 164:165, 166:167, 168:169, 170:171, 172:173, 15 174:175, 176:177, 178:179, 180:181, 182:183, 184:185, 186:187, 188:189, 190:191, 192:193, 194:195, 196:197, 198:199, 200:201, 202:203, 204:205, 206:207, 208:209, 210:211, 212:213, 214:215, 216:217, 218:219, 220:221, 222:223, 224:225, 226:227, 228:229, 230:231, 232:233, 234:235, 236:237, 238:239, 240:241, 242:243, 244:245, 246:247, 248:249, 250:251, 252:253, 254:255, 256:257, 258:259, 260:261, 262:263, 264:265, 266:267, 268:269, 270:271, 272:273, 20 274:275, 276:277, 278:279, 280:281, 282:283, 284:285, 286:287, 288:289, 290:291, 292:293, 294:295, 296:297, 298:299, 300:301, 302:303, 304:305, 306:307, 308:309, 310:311, 312:313, 314:315, 316:317, 318:319, 320:321, 322:323, 324:325, 326:327, 328:329, 330:331, 332:333, 334:335, 336:337, 338:339, 340:341, 342:343, 344:345, 346:347, 348:349, 350:351, 352:353, 354:355, 356:357, 358:359, 360:361, 362:363, 364:365, 366:367, 368:369, 370:371, 372:373, 25 374:375, or 376:377.

45. A bioagent identifying amplicon for identification of a bacterium comprising an isolated polynucleotide of about 45 to about 150 nucleobases in length produced by the process of amplification of nucleic acid encoding ribosomal RNA from a bacterium with a 30 pair of intelligent primers wherein each intelligent primer is of a length of about 12 to about 35 nucleobases, wherein the bioagent identifying amplicon provides identifying information about the bioagent.

46. The bioagent identifying amplicon of claim 45 wherein each member of the pair has at least 70% sequence identity with the sequence of the corresponding member of any one of the following intelligent primer pair sequences: SEQ ID NOs: 8:9, 10:11, 12:13, 14:15, 16:17, 18:19, 20:21, 22:23, 24:25, 26:27, 28:29, 30:31, 32:33, 34:35, 36:37, 38:39, 40:41, 5 42:43, 44:45, 46:47, 48:49, 50:51, 52:53, 54:55, 56:57, 58:59, 60:61, 62:63, 64:65, 66:67, 68:69, 70:71, 72:73, 74:75, 76:77, 78:79, 80:81, 82:83, 84:85, 86:87, 88:89, 90:91, 92:93, 94:95, 96:97, 98:99, 100:101, 102:103, 104:105, 106:107, 108:109, 110:111, 112:113, 114:115, 116:117, 118:119, 120:121, 122:123, 124:125, 126:127, 128:129, 130:131, 132:133, 134:135, 136:137, 138:139, 140:141, 142:143, 144:145, 146:147, 148:149, 150:151, 152:153, 10 154:155, 156:157, 158:159, 160:161, 162:163, 164:165, 166:167, 168:169, 170:171, 172:173, 174:175, 176:177, 178:179, 180:181, 182:183, 184:185, 186:187, 188:189, 190:191, 192:193, 194:195, 196:197, 198:199, 200:201, 202:203, 204:205, 206:207, 208:209, 210:211, 212:213, 214:215, 216:217, 218:219, 220:221, 222:223, 224:225, 226:227, 228:229, 230:231, 232:233, 234:235, 236:237, 238:239, 240:241, 242:243, 244:245, 246:247, 248:249, 250:251, 252:253, 15 254:255, 256:257, 258:259, 260:261, 262:263, 264:265, 266:267, 268:269, 270:271, 272:273, 274:275, 276:277, 278:279, 280:281, 282:283, 284:285, 334:335, 336:337, 338:339, 340:341, 342:343, 344:345, 346:347, 348:349, 350:351, or 352:353.

47. A bioagent identifying amplicon for identification of a virus comprising an isolated 20 polynucleotide of about 45 to about 150 nucleobases in length produced by the process of amplification of nucleic acid encoding a viral housekeeping gene with a pair of intelligent primers wherein each intelligent primer is of a length of about 12 to about 35 nucleobases, wherein the bioagent identifying amplicon provides identifying information about the bioagent.

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48. The bioagent identifying amplicon of claim 47 wherein each member of the pair has at least 70% sequence identity with the sequence of the corresponding member of any one of the following intelligent primer pair sequences: SEQ ID NOs: 286:287, 288:289, 290:291, 292:293, 294:295, 296:297, 298:299, 300:301, 302:303, 304:305, 306:307, 308:309, 310:311, 30 312:313, 314:315, 316:317, 318:319, 320:321, 322:323, 324:325, 326:327, 328:329, 330:331, 332:333, 354:355, 356:357, 358:359, 360:361, 362:363, 364:365, 366:367, 368:369, 370:371, 372:373, 374:375, or 376:377.

49. The method of claim 48 wherein said viral housekeeping gene is hexon, DNA-dependent polymerase, DNA-dependent RNA polymerase A, or DNA-dependent RNA polymerase B.